

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al.                      Art Unit : 1652  
 Serial No. : 09/656,309                                  Examiner : Richard Hutson, Ph.D.  
 Filed : September 6, 2000  
 Title : ENZYMES HAVING HIGH TEMPERATURE POLYMERASE ACTIVITY AND  
 METHODS OF USE THEREOF

Commissioner for Patents  
 P.O. Box 1450  
 Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Walter Callen, am a co-inventor with Eric J. Mathur, on the above-identified patent application.

2. I am an expert in the field of molecular biology and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

3. I declare that at the time of the invention it was considered routine by one skilled in the art to make multiple substitutions or multiple modifications in a nucleic acid sequence, such as a polypeptide coding sequence, and screen for functional products (e.g., variant enzymes) encoded by the modified nucleic acids. Thus, it would have been routine for one skilled in the art at the time of the invention to make multiple substitutions or multiple modifications in a polymerase coding sequence and screen for functional variant polymerase enzymes encoded by the modified nucleic acids. For example, by 1996, high through-put *in vivo*

## CERTIFICATE OF MAILING BY FIRST CLASS MAIL

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7/31/03  
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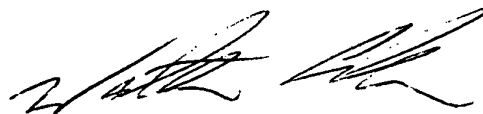
Attorney's Docket No.: 09010-027003 / DIVER1350-2

(e.g., whole cell) nucleic acid expression and screening protocols were well known in the art. In particular, high through-put methods to screen for polymerase activity, such as polymerase chain reaction (PCR), were well known in the art. Accordingly, at the time of the invention it would have been considered routine by one skilled in the art to make multiple substitutions or multiple modifications in a nucleic acid sequence and to screen for functional variations.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 7/22/03



Walter Callen



## **WALTER CALLEN**

3469 Stetson Avenue  
San Diego, CA 92122  
Email: [wcallen@diversa.com](mailto:wcallen@diversa.com)

Home: (858) 457-4633  
Work: (858) 526-5200

### **PROFESSIONAL EXPERIENCE**

Fourteen years of experience in as a molecular biologist in an industrial setting.

**Staff Scientist II** - Diversa Corporation, San Diego CA. 6/94 - present.

Responsible for the development of screening methods and discovery of novel industrial enzyme clones to fulfill partnership agreements. The overexpression of target enzymes in bacterial hosts. Radiation Safety Officer. Head of Hybridization Department. Supervisor of two technicians.

#### **Research Accomplishments:**

- Discovery of over 500 enzyme clones
- Representing over 50% of Diversa's enzyme clones
- Among these are DNA polymerases, esterases, and glycosidases
- Assisted in the screening for dehalogenases, insecticides and bioactive pathway clones
- Utilizing high-throughput screening methods

**Senior Research Associate** - Stratagene Cloning Systems, La Jolla CA. 8/89 - 6/94.

Responsible for research and development of molecular biological products. Laboratory Manager. Chemical Hygiene Officer.

#### **Research Accomplishments:**

- Cloning of six DNA modifying enzymes
- The Clearcut Miniprep kit
- The Cyclist, cycle sequencing kit
- DNA primer synthesis via Hexamer Ligation
- Kb DNA size markers
- ExoMeth sequencing kit

**Graduate Assistant** - San Diego State University, San Diego, CA. 9/85 - 8/89.

Instructor for college cell biology laboratory course and grading lab reports. Prepared and presented course materials, preparing exams and assigning grades for beginning college biology laboratory course. Responsible for ordering supplies, washing glassware, autoclaving and P1 waste disposal for molecular biology laboratory.

### **EDUCATION**

**Master of Science, Molecular Biology.** San Diego State University, San Diego, CA.

July 1989. Masters Thesis - Molecular Characterization of M308 a Flightless Mutant of *Drosophila melanogaster*.

**Bachelor of Arts, Chemistry. Bachelor of Arts, Biology** with emphasis in **Molecular Biology.** Humboldt State University, Arcata, CA. June 1985.

## **PATENTS/PATENT APPLICATIONS**

WO 02/020735 Callen, Walter; Mathur, Eric; Short, Jay. Enzymes Having High Temperature Polymerase Activity and Methods of Use Thereof.

WO 02/068589 Callen, Walter; Richardson, Toby; Frey, Gerhard; Gray, Kevin; Short, Jay; Kerovuo, Janne; Slupska, Malgorzata. Enzymes Having Alpha Amylase Activity and Methods of Use Thereof.

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AU Patent No. 735082 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

US Patent No. 6,492,511 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

US Patent No. 5,948,666 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

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## **PUBLICATIONS**

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Parker KN, Chhabra SR, Lam D, Callen W, Duffaud GD, Snead MA, Short JM, Mathur EJ, Kelly RM. Galactomannanases Man2 and Man5 from Thermotoga species: growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes. Biotechnol Bioeng 2001 Nov 75:322-33.

Sehgal AC, Callen W, Mathur EJ, Short JM, Kelly RM. Carboxylesterase from Sulfolobus solfataricus P1. Methods Enzymol 2001 330:461-71.

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Miller ES, Kimberley , Parker N, Liebl W, Lam D, Callen W, Snead MA, Mathur EJ, Short JM, Kelly RM. Alpha-D-galactosidases from Thermotoga species. Methods Enzymol 2001 330:246-60.

Chhabra S, Parker KN, Lam D, Callen W, Snead MA, Mathur EJ, Short JM, Kelly RM. Beta-mannanases from Thermotoga species. Methods Enzymol 2001 330:224-38.

Michael W. Bauer, Lance E. Driskill, Walter Callen, Marjory A. Snead. Eric J. Mathur, and Robert M. Kelly. An Endoglucanase, EglA, from the Hyperthermophilic Archaeon Pyrococcus furiosus Hydrolyzes 8-1,4 Bonds in Mixed-Linkage (1-3), (1-4)-R-D-Glucans and Cellulose. Journal of Bacteriology, 1990. Vol. 181 No.1 pg. 284-290.

K. Kretz, W. Callen, and V. Hedden. Cycle Sequencing. PCR Methods and Applications, 1994. Vol. 3 No. 5 Pg. S107.

W. Callen, V. Hedden, B. Jerpseth, J. Hayfield, J. Braman, and K. Kretz. The Clearcut Kit: A Versatile System for Rapid Plasmid Minipreps, Buffer Exchanges and PCR Cleanup. Strategies in Molecular Biology, 1993. Vol. 6 No. 2.V.

Hedden, W. Callen, J. M. Short, and K. Kretz. Improved Sequence Analysis of Mutations Identified with the Big Blue System. Strategies in Molecular Biology, 1993. Vol. 6 No.1.

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Hedden, M. Simcox, W. Callen, B. Scott, J. Cline, K. Nielson, E. Mathur, and K. Kretz. Superior Sequencing: Cyclist Exo-Pfu DNA Sequencing Kit. Strategies in Molecular Biology, 1992.Vol. 5 No. 3.

E. Marsh, W. Shoetlin, J. Cline, W. Callen, K. Kretz, and E. Mathur. Pyrococcus furiosus DNA Ligase and the Ligase Chain Reaction. Strategies in Molecular Biology, 1992. Vol. 5 No. 3.

K. Kretz, V. Hedden, and W. Callen. Cyclist DNA Sequencing Kit: Fast, Reliable Sequencing of Inconsistent Templates. Strategies in Molecular Biology, 1992. Vol. 5 No. 2.

E. J. Mathur, M. W. W. Adams, W. N. Callen, and J. M. Cline. The DNA Polymerase Gene from the Archaeobacterium, Pyrococcus furiosus, Shows Sequence Homology with Alpha-Like DNA Polymerases. Nucleic Acids Research, 1991 Vol. 19 No. 24 Pg. 6952.

L. Blinderman, W. Callen, C. Katayama. ExoMeth Sequencing: Troubleshooting Superimposed Sequences. Strategies in Molecular Biology, 1990. Vol. 3 No. 3.

J. Sorge, L. Blinderman, C. Katayama, W. Callen. ExoMeth Sequencing Method: Eliminates the Need for Subcloning and Oligonucleotide Primers. Strategies in Molecular Biology, 1990. Vol. 3 No. 1.

## ABSTRACTS

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E. Mathur, J. Cline, E. Marsh, W. Shoettlin, K. Nielson, B. Scott, W. Callen, K. Kretz, and J. Sorge. Isolation, Characterization and Cloning of DNA polymerase I and DNA ligase I from the marine hyperthermophile, *Pyrococcus furiosus*. American Society for Cell Biology Conference. Nov. '92.K.

Kretz, W. Callen, D. Shoemaker, E. Mathur, and J. Sorge. Direct Sequencing of Cosmid, Lambda, and PCR DNA Via Cycle-Sequencing with Alpha-Labeled Nucleotides. Human Genome 111, San Diego. Oct. 1991.

W. Callen, D. Shoemaker, E. Mathur, and J. Sorge. A Library of Synthetic Templates for the Directed Ligation of Any Two Oligonucleotide Hexamers. Cold Spring Harbor Human Genome Conference. April 1991.

D. Shoemaker, E. Mathur, W. Callen, J. Sorge. A Library of 256 Hexamers, Degenerate at Two Positions (5'-NNXXXX-3'), Can Create All Possible 12-mer Primers for Applications in High Volume DNA Sequencing Strategies. Cold Spring Harbor Human Genome Conference. April 1991.

W. Callen, C. Katayama, L. Blinderman and J. Sorge. Direct Sequencing of PCR Amplified DNA Bound to a Solid Support Using the ExoMeth Sequencing System. Human Genome 11, San Diego. Oct. 1990.

S.I. Bernstein, N.K. Hess, P.T. O'Donnell, K.D. Becker, D.D. Hodges, V.L. Collier, W. Callen, K. Edwards and W.A. Kronert. Analysis of Myosin Heavy Chain Isoform Function and the Mechanism of Alternative Splicing in *Drosophila*. Cold Spring Harbor Conference on RNA Processing. April 1989.

S.I. Bernstein, P.T. O'Donnell, K. Mogami, E.S. Roche, V. Collier, A. Fieck, W. Callen, W. Kronert, and E. McKennon. Molecular and Ultrastructural Analysis of Mutations in the *Drosophila* Muscle Myosin Heavy Chain Gene: Muscle-Specific Effects on Myosin Accumulation and Muscle Function. Gordon Research Conference on Molecular Genetics. August 1987.